

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



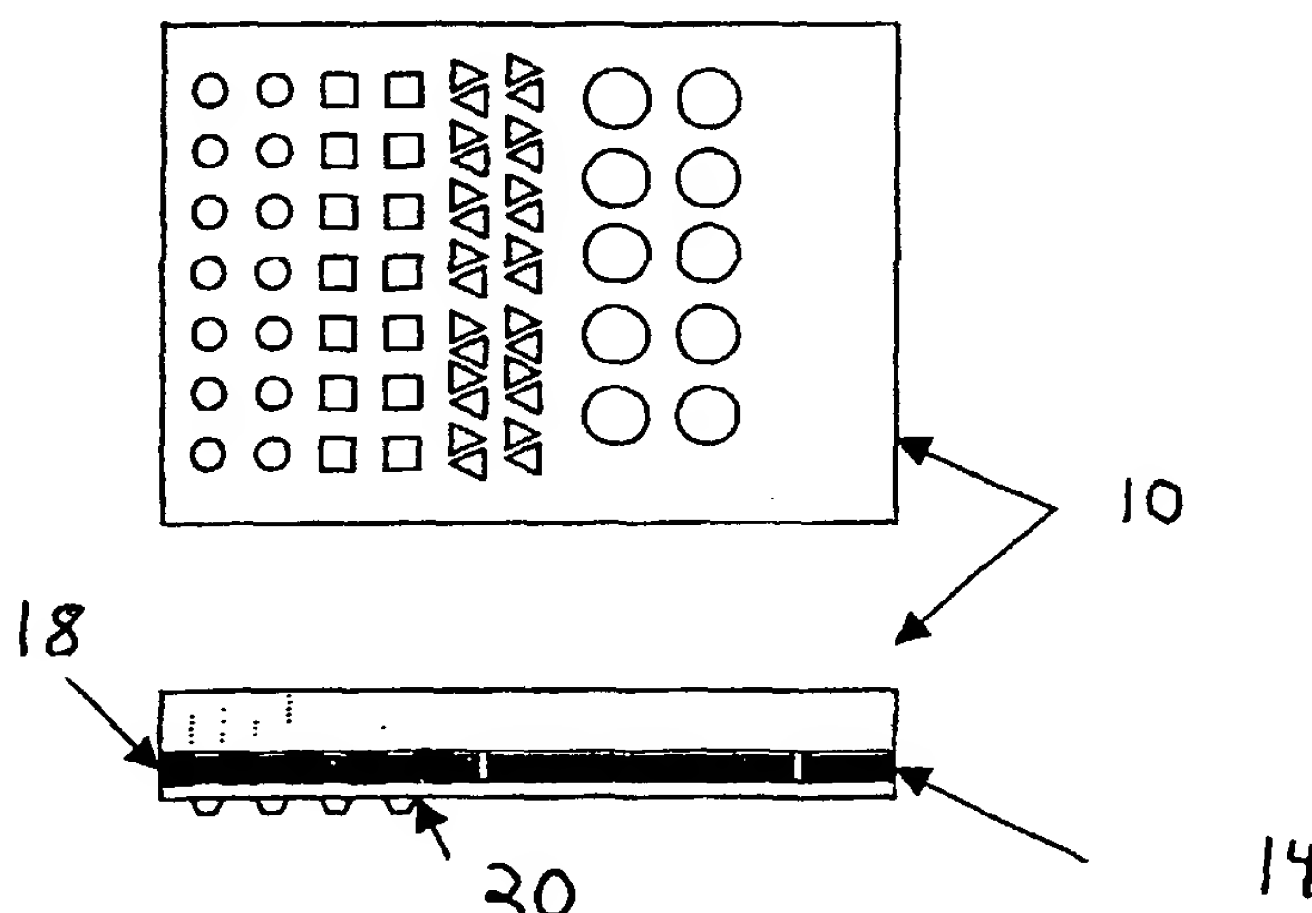
(43) International Publication Date
22 March 2001 (22.03.2001)

PCT

(10) International Publication Number
WO 01/19502 A2

- (51) International Patent Classification⁷: **B01D 63/00** (72) Inventor; and
(75) Inventor/Applicant (for US only): **FOLEY, Brian**
(21) International Application Number: **PCT/US00/25585** [US/US]; 53 Vince Brook Road, Westford, MA 01886 (US).
(22) International Filing Date: 18 September 2000 (18.09.2000) (81) Designated State (national): US.
(25) Filing Language: English (84) Designated States (regional): European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).
(26) Publication Language: English
(30) Priority Data:
✓ 60/154,565 17 September 1999 (17.09.1999) US
Published:
— Without international search report and to be republished upon receipt of that report.
(71) Applicant (for all designated States except US): **MILLIPORE CORPORATION** [US/US]; King, Timothy, J., 80 Ashby Road, Bedford, MA 01730 (US).
For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: **HIGH THROUGHPUT SCREENING CARD**



(57) Abstract: The present invention provides multi-well membrane filter and methods of producing same, the multi-well membrane filter comprising a non-injection molded support and filter membrane laminated thereto, the method particularly adapted for producing multi-well membrane filters that have a substantial well density and small volume sample wells.



WO 01/19502 A2

HIGH THROUGHPUT SCREENING CARD

Inventor: Brian Foley

5

This application relates to co-pending U.S. Applications "Three Dimensional Patterned Porous Structures," our reference number MCA-482 and "Patterned Porous Structures," our reference number MCA-474, both filed concurrently herewith.

10

BACKGROUND OF THE INVENTION

The Multiscreen® multiple well membrane filter for laboratory and research use, produced by Millipore Corporation, is used for laboratory assays. These filters and its competitive products are made by first creating a support plate by injection molding a resin. Typically, polyethylene, Barex™ polymer, acrylic or styrene is used as the resin. The support plate would have a number of through holes, with the wall of such holes serving as the side wall (circular well) or walls (multi-hedral well) of the wells. As such, the resin in its injection-molded form must not interfere with the laboratory assay it is used for.

20

The multi-well device is then created by cutting filter membrane into discs and attaching the discs to one side of each through hole, thereby creating the wells. Such filter membrane is typically attached to the support by bonding, either thermal or ultrasonic. This is a labor-intensive operation, adding substantially to the cost of manufacturing.

25

As the injection molded support plates require a mold, a mold has to be produced for each device. The process of producing a mold for use in production first requires the production of a prototype mold. Such molds cost around \$50,000. Once the final design of the product is agreed upon, a production mold is made. The cost of a production mold varies with the number of cavities intended for the injection-molding tool. For example, a one-cavity tool for 96 well plate production requires a mold that typically costs \$90,000. A two-cavity tool for 96 well plate production requires a mold that typically costs \$160,000.

30

A limitation of the molds is that they are suitable only for similar materials. One could not use the polyethylene mold to produce a styrene support. This limits the products that can be made available because it is not cost effective to produce the same plate design from two different materials due to the cost of capital and the time to recoup the investment.

For example, it would be beneficial to offer a variety of materials for supports so the researcher can avoid materials that may leach extractables into their assay. Indeed, the use of Teflon® polytetrafluoroethylene ("PTFE"), a substantially inert material, is not used to produce supports because it is not a cost effective alternative for injection molding low cost parts. For example, a PTFE multi-well plate would be useful for assays that require no protein binding by the support. Indeed, a PTFE plate in combination with a low protein binding membrane would have substantial utility. Until the present invention, such a device was not available due to its cost and the limitations of technology.

The current format of choice for multi-well membrane filters is a 96 well plate with a support that is made from injection-molded resins such as polyethylene, styrene and acrylic. 12 and 24 well formats preceded the 96 well plate, but as robotics improved and sample volumes have mushroomed, the need for more assays per plate (higher well density) has increased. Moreover, the volume of the typical assay is shrinking dramatically because of the costs of the material being assayed, proteins, nucleic acids and the like, the costs of the solvents, buffers, enzymes and the like, and improvements in detection technology. As such, that market requires multiple well membrane filters that have more wells in same area with the wells having smaller volumes. Indeed, formats of 384, 1536, 9600 wells are envisioned.

Unfortunately, the injection molding process has severe limitations beyond the current 96 well format. There are substantial manufacturing hurdles with such higher density formats such as molding (more plastic, higher piece part cost, tool building and maintenance costs) and assembly (stricter tolerances are required for cutting and sealing membrane on the wells.) Moreover, the overall size of the plate cannot

expand much, if at all, due to the customers already significant sunk costs in laboratory automation.

5 The present invention solves the prior art problems by providing a platform suitable for affixing a membrane that results in a multi-well membrane filter suitable for high throughput screening assays in high density, low volume formats. The current invention allows for quick, inexpensive change over in the manufacturing process without the expensive tooling costs required by the injection molding process. Moreover, the present invention can provide a multi-well membrane filter that is 10 much cheaper on a per well basis than prior art multi-well membrane filters. Lastly, the present invention provides a means for using PTFE supports in multi-well membrane filters that are competitive on a per well basis with the current injection molded polyethylene support in the 96 well format.

15

SUMMARY OF THE INVENTION

The present invention provides a multi-well membrane filter comprising a support characterized by through holes not having been molded therein; and a membrane filter fixed to said support so at least one side of at least two such through holes are covered such that the device has at least two wells suitable for receiving 20 material to be assayed.

25

The present invention provides a method of producing a multi-well membrane filter device, the method comprising 1) selecting a pre-formed support suitable for affixing a membrane thereto; 2) removing material from such pre-formed support so as to form substantially aligned through holes therein; 3) selecting a membrane suitable for filtering solutions in a laboratory setting; and 4) forming wells by laminating the membrane to the support.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 provides top and sectional views of a multi-well filter device with different well shapes and an underdrain laminated thereto

5

DETAILED DESCRIPTION OF THE SPECIFIC EMBODIMENT

The present invention provides a multi-well membrane filter, the filter comprising a support characterized by through holes not having been molded therein; and a membrane filter fixed to said support so at least one side of at least two such through
10 holes are covered such that the device has at least two wells suitable for receiving material to be assayed.

Preferably, the support may be made of glass, metallic materials, ceramic materials, elastomeric materials and coated cellulosic materials. In a more preferable
15 embodiment, the support includes polymeric material. Polymeric materials suitable for the present invention include polyethylene, acrylic, PTFE, polycarbonate and styrene.

Preferably, the plate height is less than one quarter inch thick. The prior made no
20 suggestion for the plate of the present invention because the volume requirements would have necessitated a plate of a large thickness, i.e., greater than one half inch. At such thickness, there is no motivation to using anything other than molding due to the costs of that process.

Preferably, the multi-well membrane filter of the present invention is configured to have at least 96 wells. In such a configuration, wells in a specific device may have different volumes. Preferably, individual wells have a volume in the range of 50 to 150 microliters. More preferably, the individual wells have a volume in the range of 70 to 130 microliters. For the 384-well format, a volume of about 100 to 120
25 microliters is preferred. It is envisioned that for formats greater than the 384-well
30 format, the volume requirements will diminish. The wells may also have different shapes.

In a preferred embodiment, the multi-well membrane filter of the present invention will have an underdrain laminated to the opposite side of the membrane to facilitate collection of filtrate.

- 5 In a preferred embodiment of the present invention, the membrane contains patterned porous structures.

The present invention provides a method of producing a multi-well membrane filter device, the method comprising selecting a pre-formed support suitable for affixing a
10 membrane thereto; removing material from such pre-formed support so as to form substantially aligned through holes therein; selecting a membrane suitable for filtering solutions in a laboratory setting; and forming wells by laminating the membrane to the support.

- 15 Preferably, the method of the present invention includes extruding a material of -interest to form the pre-formed support.

Preferably, the through holes in the pre-formed support are made by selectively
20 drilling, punching, burning or dissolving material to be removed.

The preferred method of laminating the membrane to the support is in a web converting process. Preferred methods of laminating include diffusion bonding, adhesive bonding, welding and thermal bonding.

- 25
Figure 1 provides an example of the present invention. A rigid sheet 10 is infused with a matrix of wells. The wells could be pre-punched or they could be produced online within an assembly process, such as a web converting process. For example, the perforated sheet could be presented to the assembly machine precut and stacked or
30 on a continuous web and cut to size online. The well matrix will be determined by the end users needs, but it could have numerous configurations and the wells do not necessarily need to be all of the same shape or size. Figure 1, Top View, provides the

wells of the present invention having round, square, and other shapes. Virtually any shape that is required for the product may be provided.

5 The rigid sheet 10 allows for easy handling during manufacturing and easy handling during use by the end user (a human or a robot). As the web process uses sheet stock, using sheet/roll stock as the starting material allows for a variety of resins and support thicknesses because most vendors of sheet stock supply a wide range of resins and thicknesses to be used with this type of assembly process. By varying the thickness of the sheet, various products could be developed with different starting well volumes.

10 One could also use the same sheet thickness and vary the volume by removing more material when the through holes are created.

After the perforated sheet is produced with the appropriate array, a membrane 14 is attached to the perforated sheet. The membrane could be completely hydrophobic or

15 hydrophilic or it could be hydrophilic with hydrophobic regions 18.

The present invention may also be used in connection with related case no. MCA-474 filed concurrently herewith that is related to porous structures having selected functional patterns upon and/or in them. That invention is particularly related to

20 porous structures such as membranes that have a series of one or more patterns of porous and non-porous areas. Such patterned porous structures may be laminated on the support structure of the present invention. It is envisioned that non-porous regions of the membrane will be situated where the hydrophobic region 18 is in Figure 1.

25 A variety of laminating process is envisioned, yet the below description is not exclusive. Those of ordinary skill in the art would appreciate other means of attaching two layers together like those described herein.

The means of laminating; that is, attachment could be any of the following:

30 1. Heat (thermal, ultrasonic, vibration)- The membrane and perforated sheet are held together under pressure. Then ultrasonic energy, vibration energy, or thermal energy applies heat. The melted resin will be pushed into the pores of the membrane.

2. Epoxy- A very thin film of epoxy (air cured, heat cured, UV cured) is applied to 1 side of the perforated sheet. Then the membrane is applied and held in place at specific pressure and cured. If UV is used for curing, then the sheet and or the membrane must be transparent to the UV light. Although epoxy is discussed in this disclosure, most adhesives (silicone based, acrylic based, etc.) will work as described above as long as they are compatible with the rigid sheet, membrane and assay.
3. Solvent- A solvent that dissolves the perforated sheets only is applied to 1 surface of the perforated sheet or to the membrane. The membrane is pressed onto the perforated sheet and held until the excess solvent has been dispersed. The dissolved resin will be pushed into the pores of the membrane. In another method, a solvent that can dissolve both the perforated sheet and the membrane is applied only to the perforated sheet. The membrane is pressed onto the perforated sheet and held until the excess solvent has been dispersed. In this method the dissolved resin from the membrane and the dissolved resin from the perforated sheet mix and solidify. For example, Durapore® membrane produced by Millipore Corporation, would be solvent bonded to a polycarbonate sheet with methylene chloride. It is critical that the solvent be compatible with the materials used to produce the support and the membrane.
4. Non-contact fusion bonding- The surface of the perforated sheet is heated to it's melting point. The heat source is quickly removed and the membrane is pressed and held until the resin solidifies.
5. Diffusion bonding- The membrane and perforated sheet are pressed together at a "critical" temperature and pressure. At the critical point the resins will molecularly mix and solidify.

The present invention also provides a process of making a multi well membrane with limited cross talk. Specifically the process further comprises the step of making the seal formed around the individual wells after the lamination step impervious to the filtrate.

In applications where filtrate collection is not required, the seal between the support and the membrane needs only to be secure enough to prevent the membrane from releasing from the sheet and prevent the fluid from crossing from well to well between the sheet and the surface of the membrane. In applications that require filtrate
5 collection, it is imperative that the seal formed around the individual wells is impervious to the filtrate so that cross talk between the wells is avoided.

The present invention provides a variety of different means of making the seal formed around the individual wells after the lamination step impervious to the filtrate.

10

1) Collapsing the pores in the membrane with heat and prior to laminating to the support. Collapsing the pores could possibly be done at the same time as laminating.

15 2) After laminating, break or remove membrane from between the wells by die cutting, ultrasonic vibration or heat.

3) Using a hydrophobic barrier pre or post laminating.

20 4) Filling the non-well forming membrane pores with epoxy.

5) Using solvents that will solvate the sheet and the membrane, thereby collapsing the pores in non-well forming membrane.

25 6) Using solvents that will dissolve only the rigid sheet. This will fill the pores in the non well-forming membrane with material from the rigid sheet.

The Sectional View of Figure 1 presents the use of an underdrain 20 with present invention. Underdrains are typically vacuum formed or molded and may be added
30 after the membrane has been laminated it may added at same time as the membrane. The same bonding technologies described above would be used during lamination of the underdrain 20.

The present invention results in a superior multi-well membrane device process because the manufacturing cost thereof on a well-to-well basis will be much less expensive than prior art because the support or rigid sheet will be less expensive than a molded part.

5

The present invention results in a superior multi-well membrane device process because the assembly process could be a web converting process. Use of such process would further reduce assembly costs.

- 10 The present invention results in a superior multi-well membrane device process because multiple resins and thickness are readily available for the rigid sheets. Moreover, product design variations (chemical compatibilities, geometries, thicknesses and the like) can be made with minor die punch changes (this cannot be done with injection molds) and minor adjustments to the assembly equipment.

15

The present invention results in a superior multi-well membrane device process because the rigid sheet concept allows for multiple line extensions using the same assembly equipment.

- 20 The present invention results in a superior multi-well membrane device because it provides substantially increased well density, lower volume, tailored volume in a single card and the ability to use PTFE on a cost effective basis.

- 25 The above examples are not to be construed as limiting the scope of the subject invention, which is set forth in the claims below. Those of ordinary skill in the art will appreciate the types of membranes appropriate to practice the present invention.

CLAIMS

1. A multi-well membrane filter, the filter comprising:
a support characterized by through holes not having been molded therein; and a
membrane filter fixed to said support so at least one side of at least two such
through holes are covered such that the device has at least two wells suitable for
receiving material to be assayed.
2. The multi-well membrane filter of claim 1, wherein the support includes glass.
3. The multi-well membrane filter of claim 1, wherein the support includes metallic
materials.
4. The multi-well membrane filter of claim 1, wherein the support includes ceramic
materials.
5. The multi-well membrane filter of claim 1, wherein the support includes
elastomeric materials.
6. The multi-well membrane filter of claim 1, wherein the support includes coated
cellulosic materials.
7. The multi-well membrane filter of claim 1, wherein the support includes a
polymeric material.
8. The multi-well membrane filter of claim 2, wherein the polymeric material
includes polyethylene.
9. The multi-well membrane filter of claim 2, wherein the polymeric material
includes acrylic.

10. The multi-well membrane filter of claim 2, wherein the polymeric material includes PTFE.
11. The multi-well membrane filter of claim 2, wherein the polymeric material includes polycarbonate.
12. The multi-well membrane filter of claim 2, wherein the polymeric material includes styrene.
13. The multi-well membrane filter of claim 1, wherein the support and membrane are configured to have at least 96 wells.
14. The multi-well membrane filter of claim 13, wherein the support and membrane are configured to have at least 384 wells.
15. The multi-well membrane filter of claim 1, wherein at least two wells have different volumes.
16. The multi-well membrane filter of claims 1 and 14, wherein the volume of each well is in the range of 50 to 150 microliters.
17. The multi-well membrane filter of claim 16, wherein the volume of each well is in the range of 70 to 130 microliters.
18. The multi-well membrane filter of claim 1, wherein at least two cells have different shapes.
19. The multi-well membrane filter of claim 1 further comprising an underdrain laminated to the opposite side of the membrane.
20. The multi-well membrane filter of claim 1, wherein the membrane contains patterned porous structures.

21. A method of producing a multi-well membrane filter device, the method comprising:
- selecting a pre-formed support suitable for affixing a membrane thereto;
 - removing material from such pre-formed support so as to form substantially aligned through holes therein;
 - selecting a membrane suitable for filtering solutions in a laboratory setting; and
 - forming wells by laminating the membrane to the support.
22. The method of claim 21 further comprising extruding a material to form the pre-formed support.
23. The method of claim 21, wherein material is removed from the pre-formed support by selectively drilling out material to be removed.
24. The method of claim 21, wherein material is removed from the pre-formed support by selectively punching out material to be removed.
25. The method of claim 21, wherein material is removed from the pre-formed support by selectively burning material to be removed.
26. The method of claim 21, wherein material is removed from the pre-formed support by selectively dissolving material to be removed.
27. The method of claim 21, wherein the membrane is laminated to the support in a web converting process.
28. The method of claim 21, wherein the membrane is laminated to the support by diffusion bonding.
29. The method of claim 21, wherein the membrane is laminated to the support by adhesive bonding.

30. The method of claim 21, wherein the membrane is laminated to the support by welding.
31. The method of claim 21, wherein the membrane is laminated to the support by thermal bonding.
32. The method of claim 21 further comprising the step of making the seal formed around the individual wells after the lamination step impervious to the filtrate.

Figure 1

